Comments received and Topic Leader responses on the August 11, 2000 draft (f) of the VICH Mycoplasma Guideline.

March 23, 2001

Section 1.3

JMAFF - "chicken embryo origin" is better to revised by "chicken embryonated egg origin".

Topic Leader (TL) Response - Change made. A common wording to describe these products will be inserted. We need to be sure it is clear those products made in embryonated eggs must also be tested for the presence of Mycoplasma as well as those products made in cell culture.

Section 2.1

NZMAF - Sampling regimes should be amalgamated and revised for added clarity as described here:

Samples should be taken at the following stages and tested for the presence of Mycoplasma as follows:

A) Master seeds and master cells, and

B) Working seed and cell lots derived from them, and

C) Pooled bulk harvest, or where the harvest comprises a single batch of that harvest, or

D) Final containers of each batch which has not been tested for Mycoplasma at the harvest or pooled harvest stages.

TL Response – Change made. This is an example of how this paragraph will read after the table "Segments of the Vaccine Production Process Required by Regulations to be tested for Mycoplasma Contamination: By Country and Test Method" is completed and the working group (WG) agrees which stages will be tested according to this guideline. The TL hopes the WG can agree to the stages via e-mail so that this guideline may be finalized at the next WG meeting in Tokyo.

Section 2.2

JMAFF - About PCR method, the item which "PCR method is an useful method, and it will be adopted as test method insert that is promising" will be instead in a suitable point, and do not mention an item in a guideline especially. And, in the August draft, art type of PCR method mentioned in an appendix eliminates it.

JMAFF - In the guidelines, I agree to an opinion to raise as an examination subject toward introduction of the future of the PCR method. But the various examinations that applied PCR method currently in each country are enforced and do it and enforce accumulation of data toward the future tentatively in each country. If the PCR method will remark to the appendices for a purpose to accumulate the data, we must add a postscript to a method with possibility adopted as quality control method in the future, and mention in detail. And should not we mention a more detailed method in order to compare it, and to examine it? On the basis of PCR method which Japan proposed, the modified PCR method is mentioned in appendices. We require to mention the PCR method written in the drug standard, which we had been shown in a 1st WG conference, precisely (for example, materials, reaction solution). As avoid the misunderstanding, it is thought with uselessness in a current guideline to an additional note of modified PCR

method. We include comparison examination about a selection of PCR method considered to be suitable or confirmation by a joint study, and, toward introduction, argument of most will become necessary in future.
JMAFF - In the present, PCR method is not adopted in a system of regulation with 3 regions. Utility of PCR method is completely understood, but more investigations are necessary for the application. In Mycoplasma symposium held by sponsorship of EDQM, it was confirmed useful as detection tool of Mycoplasma. In other words, it is strong technique for field survey and research exploitation of PCR methods. The fact is understood from much reports related to including Mycoplasma. However, it is unfamiliarity that adopt as specification detection method of biologicals and it is task after now. Even if it is one written with a clause of appendix instead of mention of guideline body, it is guideline of VICH. I insisted as Japanese regulation representative that we should have avoided a mention of detailed technology about PCR method as detection method for biologics. We should discuss it about choice of the best examination method when judged that it is suitable to mention in guideline. I consulted all WG members in the end of September and submitted Progress reports in the VICH secretariat. I think that it is not suitable to remarkable difference exists in the mention content and Minutes. I want to suggest it to you. We will make the last conclusion in next Tokyo meeting about the right or wrong of acceptance to guideline of this PCR method. Therefore you do a mention as following to a suitable part of minutes of Ames meeting. 1) It was evaluated that PCR method was method to be useful in detection of Mycoplasma. 2) However, a clear mention about PCR method is not found in current standard detection method in 3 regions. 3) And we avoided a reference about methodology of PCR method at present so that there was necessity of development as detection method of biologicals. This issue should intensively discuss it in next meeting . . . this task is settled with next meeting and thinks that continuous discussion should not intend for it (It is for my the final suggestion to add "Continuous analysis is necessary" to title of PCR).
TL Response – Change made. We understood that the WG felt that the PCR method should be included in the guideline, not as required method but as a suggested additional method. The WG felt that additional data was needed concerning the usefulness of PCR in vaccine testing and by including this method in the Appendix the accumulation of this data would be fostered. On further discussion it is clear the entire WG did not agree to this, and so the section has been deleted and the issue added to the “To Be Determined” list.

Section 2.3
EU Pharm - Despite what we say in the present EP test, Acholeplasma laidlawii is not the best species for detecting interference by the antibiotics usually used in vaccines in Europe (but may be for Japan/USA?). Mycoplasma orale is better (see data presented in Paris). A. laidlawii should be used routinely since it is one of the most common contaminants and it seems that it can be of environmental origin. I believe that its growth requirements are also slightly different from the other species proposed. M. orale can come from the human operators in the factory and its use for all products seems advisable.
FEDESA – agree … is using data that MicroSafe presented in Paris.

TL Response: No change made, addressed in previous draft of guideline.

Section 2.3.2 sentence 1

JMAFF - When the broth culture medium incubate, the new condition in microaerophilic condition (the nitrogen gas existence lower part that 5-10% include CO_2) is added. We want to know the reason and significance. It was thought that there is not inevitability to add this condition to culture of a liquid nutrient medium. It is in the contents of the August draft for the first time, and this revised reason is indistinct and is proper as a mention method of materials.

EU Pharm – The guideline leaves a choice between air/microaerophilic for incubation of liquid media. We need advice from specialists about this but it may be acceptable. Plates must be incubated in microaerophilic conditions (which is now specified in the guideline).

FEDESA - Our experience with Liquid media, indicates to us that the conditions choosen for incubation aerobic v microaerophillic do not influence the growth of mycoplasmas to any significant degree, A column of liquid medium of the viscosity of mycoplasma broth will provide a range of oxygen tensions through out the column. Ranging from microaerophillic deep in the medium to aerobic at the top of the column. One point of caution is that when the media are incubated in an enriched CO2 atmosphere the top of the container should be firmly closed, as ingress of CO2 can lead to acidification of the medium and a false positive colour change.

TL Response – No change made. We understood through comments received and WG discussions that the different gaseous environments under which the flasks of liquid media were incubated had no affect on Mycoplasma growth. Therefore the guideline was changed previously to allow either atmospheric condition.

Section 2.3.2 sentence 2

JVBA - We incubate all agar plates in air and 5% CO_2 and appropriate humidity. Is this condition unacceptable? Do the literatures or reports providing the reason why microaerophilic (nitrogen containing 5-10% CO_2) condition should be used have been received?

TL Response – No change made. We understood the WG decided to include only one incubation condition in the guideline and that the European and US government laboratories had experienced better growth with microaerophilic when the 2 conditions were compared, both in the number of isolates found and CFUs detected. One literature reference is; "Optimal temperature and atmospheric conditions for growth", by R.S. Gardella and R.A. DelGuidice, in Methods in Mycoplasmology, Vol 1, edited by S. Razin and J.G.Tully.

Section 2.3.3

JVBA - We are thinking that if each new lot (batch) of media must be tested for nutritive properties of the working references in Section 2.3.1, it is not necessary to include "or whenever new lots of media ingredients are incorporated".

TL Response – Change made. We agree it is not necessary to test whenever new lots of ingredients are incorporated if "every" batch of media is tested.

Section 2.3.4 (first and second sentences)
*NZMAF - presence **and absence** (first sentence)*
*AHI – 'formulation **significantly** changes' (first sentence)*
*NZMAF – subcultured from the broth **containing product, and are present on the agar plates sub-cultured from the broths containing no product,** (second sentence)*
*NZMAF – e.g, **this may be achieved** by dilution in a larger volume (second sentence)*
TL Response – Changes made. All wording changes will be included to add clarity, except "significantly changes" because the ingredients in a vaccine product which cause inhibition of Mycoplasma are unknown.

Section 2.3.4 (second sentence)
EU Pharm - The guideline concludes that inhibition is present if the test strain fails to grow, but reduced growth would also indicate inhibition. At the moment it would be best to state that if there is notable reduction then inhibition has occurred. The definition of 'notable reduction' could be refined when we have experience with the variability of the count for the reference strains.
FEDESA – I think that this (inhibition) *is the most difficult to define. Our interpretation is as follows: The delay in growth between the positive control and the test substance should not be more than one passage. e.g If the positive control sub-culture shows growth on the plates at day 3 of incubation, then the test substance culture must show growth on the plates at day 7 equivalent to that of the positive control plates, to be considered free of inhibitory substances.*
TL Response – No change made. The method of determining inhibition will be included in the "To Be Determined" topics.

Section 2.3.5.1 line 1
EU Pharm - The two laboratories contacted confirmed the value of this (plate inoculation on day 0) *since occasionally a sample is found positive only with direct inoculation. This confirms the data distributed by USDA at Ames (103 positives from 3209 subcultures, 1 only at day 0).*
FEDESA – Definitely in agreement, heavily contaminated samples are often positive on direct inoculation, and timely results helps contamination control within the production facility.
TL Response – No change made. Using a 0 day plate, contamination can be detected in 7-10 days rather than 10-13, so elimination of contamination can start earlier, limiting consequences. Issue included in "To Be Determined" topics.

Section 2.3.5.1 line 2
AHI – (Volume of medium) *. . . will depend on inhibitory substance validation*
JMAFF - The opinion of AHI is a problem of "quantity of inoculation", and it is necessary to change a quantity of nutrient medium? It is not practical that we do a quantity of nutrient medium than 100mL in each test. If we want to deteriorate the influence of restraint supplies by dilution, it is thought that a quantity of inoculation change is practical.
TL Response – Change made. Inoculation volume should not be reduced, but inhibition must be overcome by dilution. The guideline will be changed to read "at least 100 ml".

Section 2.3.5.1 line 6
EU Pharm - The following data on the frequency of positives between 14 and 21 days in broth is from a contract testing laboratory that carries out about 2000 tests per year. About 0.3 % are positive (6 of 2000) and half of those (3 of 2000) are found only between 14 and 21 days. The late-positive samples are typically from vaccine production in-process control and appear to be associated with the presence of antibiotics. Late-positives have not been found in samples from R & D labs (usually cell cultures): positives are more frequent in such samples but are always found early. The frequency of late positives supports the 21-day broth incubation.
FEDESA - . . . is using data that I sent . . .on MicroSafes experiences with late positives, we have observed that positives in final products are rare events but often appear late. We attribute this to the presence of antibiotic residues from the cell culture phase. As such I believe that a 21 day incubation period with a final sub-culture performed from the broth on day 21 is advisable.
TL Response – No change made. Data confirms 21 day broth incubation included in guideline. Copy of data, reprint, or proceedings for distribution to WG would be helpful.

Section 2.3.5.2
*AHI - . . . if any of the plates inoculated before **6th not 7th** day are broken . . .*
TL Response – Change made. Guideline will be changed to read "6th or 7th".

Section 2.3.5.3
NZMAF - Inconsistencies in the use of medium and media. Medias should never be used)
TL Response – Change made. Inconsistencies will be corrected.

Section 2.4
JVBA – better to describe "or other equivalent in efficiency" because better cell than VERO may be found in future.
TL Response – Change made. The language will be inserted and VERO deleted as specific identifier.

Appendix B
NZMAF New Zealand - Given the criteria proposed for the acceptability of the positive control Mycoplasma references (100 CFU ± 30), the fact that different suggested media are proposed for use in the three regions, and that the master references are issued by one of the regions, we believe there will be a requirement for adaptation of the references in the regions receiving the masters to the media used in the regions, to assure their performance against the criteria set. However we note at the end of the first paragraph of section B, "A group of laboratories in the 3 regions will standardize these references and validate the CFUs." Is this intended to refer to the process we are refering to? It is unfortunate that the working group is unable to standardize the media to be used across the three regions.)
JMAFF - As having argued by a Tokyo conference, we understand to difficult to unify a medium because of a problem of addition thing. Therefore we agreed to a medium using

in the each laboratory. We examine setting of reference stock currently in order to evaluate each medium.
On sorting of reference stock, distribution method, origin of distribution, culture method, we must argue and build in it in a guideline in future.
TL Response – No change made. Due to the complexity of the Mycoplasma media formulations the working group thought there would be great difficulty in requiring several specific standard media (most members felt that more than 1 medium would be required to grow all the reference strains at the required sensitivity, 100 CFU). The working group instead has proposed a system in which standard reference strains would be produced which contain low numbers of Mycoplasma. The testing laboratory must prove their proficiency by utilizing whatever media necessary to isolate the required reference strains(see section 2.3.1). Adaptation of media may be required to obtain standardized results. The providers of the references will need to gather and share data to determine if adaptation of the references in the regions will become a problem. The trial study between the laboratories of the 3 regions would optimally be conducted during the comment period for the guideline. The study method is included in the list of issue to be determined.

General
JVBA - It would be helpful to insert the phrase (to be determined) in the appropriate part of a sentence within the guideline when matters have not yet been decided.
JMAFF - It is better to distinguish between the matter which decided a draft of guideline in a WG conference of No. 3 on making and a matter of no-decision or no-discussion definitely and mention it. About a matter of no-discussion, it is better to do an underlined mention and specify what the matter must be discussed in future, in the same way as last draft. And I understand that the matter which EU insists will be examined about the inevitability and replied it for WG, and for the swiftness nature of deliberation in future, it is better to mention clearly. For example, incubation condition and time, inoculate volume, action of inhibitory substrate, adjustment of pH, etc.
JMAFF - An opinion of a leader is that the matter must be decided in future is [a sample] holds big specific gravity, but I argue in future, and an agreement seems to be provided by a Tokyo conference, and it is thought that it is the point that it argues, and must go in the face that was made in order to surely grasp the situation of each country about this matter by the cause. But it is thought that the matter which is still left in addition to this point exists. It will be necessary for a marking to do what we talked about by now, the thing that are still undecided when I lose an argument point toward a Tokyo conference definitely.
TL Response – Change made. We agree that noting issues to be determined in the discussion draft guideline is useful. After the third WG meeting in Ames only one major part of the guideline was left "to be determined" and that is section 2.1 Samples. The WG decided that the TL should attempt to complete this section on sampling procedure, via e-mail, utilizing a table to be filled in by all members of the WG. From the completed table the TL will propose the first draft of section 2.1. WG members will then comment on this proposed 2.1 Samples section, and a workable compromise will be reached. This compromise will become part of the guideline to be completed in Tokyo. Other issues "To Be Determined" have been identified and marked in the guideline.